CLAIMS

What is claimed is:

1. A method for DNA synthesis at high pH, comprising: a) providing a DNA polymerase fusion; and contacting said fusion with a nucleic acid template, wherein said fusion permits DNA synthesis.

2. The method of claim 1, further comprising a PCR enhancing factor and/or an additive.

3. A method for cloning of a DNA synthesis product at high pH comprising:

a) providing a DNA polymerase fusion;

b) contacting said fusion with a nucleic acid template, wherein said fusion permits DNA synthesis to generate a synthesized DNA product; and

c) inserting said synthesized DNA product into a cloning vector

4. The method of claim 3, further comprising a PCR enhancing factor and/or an additive.

5. A method for sequencing DNA at high pH, comprising the steps of:

(a) contacting a template DNA strand with a sequencing DNA primer;

(b) contacting said DNA of step (a) with a DNA polymerase fusion, deoxyribonucleoside triphosphates, and a chain-terminating nucleotide analog;

(c) incubating the mixture of step (b) under conditions sufficient to synthesize a random population of DNA molecules complementary to said first DNA molecule, wherein said synthesized DNA molecules are shorter in length than said first DNA molecule and wherein said synthesized DNA molecules comprise a terminator nucleotide at their 5′ termini; and

(d) separating said synthesized DNA molecules by size so that at least a part of the nucleotide sequence of said first DNA molecule can be determined.

6. The method of claim 5, further comprising a PCR enhancing factor and/or an additive.

7. A method of linear or exponential PCR amplification at high pH for site-directed or random mutagenesis comprising the steps of: incubating a reaction mixture comprising a nucleic acid template, at least one PCR primers, and a DNA polymerase fusion under conditions which permit amplification of said nucleic acid template by said fusion to produce a mutated amplified product.

8. The method of claim 7, further comprising a PCR enhancing factor and/or an additive.

9. A method of reverse transcriptase PCR at high pH comprising the steps of incubating a reaction mixture comprising a nucleic acid template, at least one PCR primer, and a DNA polymerase fusion under conditions which permit amplification of said nucleic acid template by said fusion to produce an amplified product.

10. The method of claim 9, further comprising a PCR enhancing factor and/or an additive.

11. The method of claim 1, 3, 5, 7 or 9 wherein said DNA polymerase fusion has reduced DNA polymerization activity.

12. The method of claim 11, wherein said DNA polymerase fusion comprises a Glycine to Proline substitution at amino acid position 387 (G387P) and has reduced DNA polymerization activity.

13. The method of claim 1, 3, 5, 7 or 9, wherein said DNA polymerase fusion comprises reduced base analog detection activity.

14. The method of claim 13, wherein said DNA polymerase fusion comprises reduced base analog detection activity and a mutation at position V93, wherein said mutation is a Valine to Arginine substitution, a Valine to Glutamic acid substitution, a Valine to Lysine substitution, a Valine to Aspartic acid substitution or a Valine to Asparagine substitution.

15. The method of claim 11 wherein said DNA polymerase fusion has reduced base analog detection activity.

16. The method of claim 12, wherein said DNA polymerase fusion comprises reduced base analog detection activity.

17. The method of claim 11, wherein said DNA polymerase fusion further comprises a mutation at position V93, wherein said mutation is a Valine to Arginine substitution, a Valine to Glutamic acid substitution, a Valine to Lysine substitution, a Valine to Aspartic acid substitution or a Valine to Asparagine substitution that confers a reduced base analog detection activity phenotype to said chimeric DNA polymerase.

18. The method of claim 12, wherein said DNA polymerase fusion further comprises a mutation at position V93, wherein said mutation is a Valine to Arginine substitution, a Valine to Glutamic acid substitution, a Valine to Lysine substitution, a Valine to Aspartic acid substitution or a Valine to Asparagine substitution that confers a reduced base analog detection activity phenotype to said chimeric DNA polymerase.

19. The method of claim 13, wherein said DNA polymerase fusion further comprises a reduced DNA polymerization activity.

20. The method of claim 14, wherein said DNA polymerase fusion further comprises a reduced DNA polymerization activity.

21. The method of claim 13, wherein said DNA polymerase fusion further comprises a Glycine to Proline substitution at amino acid position 387 (G387P) that confers a reduced DNA polymerization phenotype to said chimeric DNA polymerase.

22. The method of claim 14, wherein said DNA polymerase fusion further comprises a Glycine to Proline substitution at amino acid position 387 (G387P) that confers a reduced DNA polymerization phenotype to said chimeric DNA polymerase.

23. The method of claim 1, 3, 5, 7 or 9, wherein said DNA polymerase fusion further comprises an Aspartate to alanine substitution at amino acid 141 (D141A) and a Glutamic acid to Alanine substitution at amino acid position 143 (D141A/E143A) that renders said chimeric DNA polymerase 3'-5' exonuclease deficient.

24. The method of claim 13, wherein said DNA polymerase fusion with reduced base analog detection activity further comprises an Aspartate to alanine substitution at amino acid 141 (D141A) and a Glutamic acid to Alanine substitution at amino acid position 143 (D141A/E143A) that renders said chimeric DNA polymerase 3'-5' exonuclease deficient.

25. The method of claim 1, 3, 5, 7 or 9, wherein said DNA polymerase fusion comprises a wild type, mutant or chemically modified DNA polymerase.

26. The method of claim 1, 3, 5, 7, or 9 wherein said DNA polymerase fusion is a proofreading polymerase.

27. The method of claim 26, wherein said proofreading polymerase is selected from the group consisting of Pfu, KOD, Tgo, Vent and DeepVent.

28. The method of claim 1, 3, 5, 7 or 9, wherein said DNA polymerase fusion further comprises a polypeptide with at least one of an increase in an activity selected from the group consisting of: processivity, proofreading, fidelity, DNA binding activity, strand displacement activity, polymerase activity, nucleotide binding and recognition, efficiency, template length amplification capability, GC-rich target amplification efficiency, specificity, thermostability, intrinsic hot start capability, or salt resistance.

29. The method of claim 1, 3, 5, 7 or 9, wherein said DNA polymerase fusion further comprises a polypeptide with at least one of a reduced activity selected from the group consisting of: DNA polymerase activity at room temperature, amplification slippage on templates with tri-nucleotide repeat stretches, extension time in a PCR reaction or amplification cycles in a PCR reaction.

30. The method of claim 1, 3, 5, 7 or 9 wherein said DNA polymerase fusion consists of a protein domain selected from the group of : thioredoxin processivity factor binding domain of bacteriophage T7, archaeal PCNA binding domain, PCNA, the helix-hairpin-helix DNA binding motifs from DNA topoisomerase V or the DNA binding protein Sso7d or Sac7d.

31. A kit for performing at high pH a method selected from the group consisting of: DNA synthesis; cloning of a DNA synthesis product; sequencing DNA; RT PCR; and linear or

exponential PCR amplification comprising a DNA polymerase fusion and packaging materials therefore.

32. The kit of claim 31, further comprising a high pH buffer.

33. The kit of claim 31, further comprising a PCR enhancing factor and/or an additive.

34. A composition for any one of DNA synthesis, cloning of a DNA synthesis product at high pH, sequencing DNA, linear or exponential PCR amplification for site directed or random mutagenesis, RT-PCR comprising a DNA polymerase fusion and a high pH buffer.

35. A composition for DNA synthesis, comprising a DNA polymerase fusion and a high pH DNA synthesis buffer.

36. A composition for cloning of a DNA synthesis product, comprising a DNA polymerase fusion and a high pH DNA cloning buffer.

37. A composition for sequencing DNA, comprising a DNA polymerase fusion and a high pH DNA sequencing buffer.

38. A composition for linear or exponential PCR amplification for site directed or random mutagenesis, or for RT-PCR comprising a DNA polymerase fusion and a high pH PCR reaction buffer.

39. The composition of claims 34, 35, 36, 37 or 38, further comprising a PCR enhancing factor and/or an additive.